

Final Project Evaluation Report

We ask all grant recipients to complete a project evaluation that helps us to gauge the success of your project. This must be sent in **MS Word and not PDF format**. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Complete the form in English and be as concise as you can. Note that the information may be edited before posting on our website.

Please email this report to jane@rufford.org.

Your Details	
Full Name	Daniele de Almeida Miranda
Project Title	Perfluoroalkylated compounds in tropical estuaries: degradation, biomagnification and effects on the benthic community
Application ID	2992-1
Grant Amount	£5000
Email Address	danielemirandaba@gmail.com
Date of this Report	August 03, 2018

1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
1) Evaluate the degradation processes of sulfluramid in situ				All samples were collected, the extraction procedure (that included six steps) was done and the samples were analysed by Stockholm University. The data is being analysed.
2) Verify the bio-magnification of PFASs in tropical estuaries				All samples were collected at Subaé, the extraction procedure was done the extraction procedure (that included six steps) was done and the samples were analysed by Stockholm University.
3) Evaluate deleterious effects that sulfluramid degradation products cause in the macrobenthic biota				Because of the effort to achieve the goals one and two, there was no time enough and financial recourses to work on this objective, which necessity the conclusion of the objective one and two to better planning and performer this goal.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled.

To achieve the main goals of the project, a vast human effort needed to be applied. First, to performed the objective one (evaluate the degradation processes of sulfluramid in situ, figure 1), an in situ experiment of 192 days (three times more than what was planned) were proceeded, resulting in 83 sediment samples and 16 root samples to analyse grain size, organic matter and perfluoroalkylated compounds. The days of experiment were increased to follow all process of sulfluramid degradation.

The experiment (authorisation for scientific activity issued by SISBIO: n° 57866-1) was carried out in 10 sampling sites, six areas being artificially contaminated with sulfluramid formicide (impacted areas with and without root) and four areas without sulfluramid (control areas with and without root), distant from each other by approximately 1 m. The areas were delimited by inserting a PVC tube 20 cm deep and 15 cm in diameter into the sediment of the mangrove. For the impacted areas, 2 kg of sediment were removed from the interior of each PVC tube and 500 g of the

formicide bait sulfluramid were mixed. Then the homogenized sediment was returned to the tubes. The same procedure was repeated for the six impacted areas. Samples were collected eight times, one time before contamination (T1: 0d); and seven times after addition of sulfluramid in the mangrove sediments (T2: 2d, T3: 15d, T4: 30d, T5: 60d, T6: 90d, T7: 150d, T8: 180d). The grain size determination and moisture percentage were obtained separately by the gravimetric method and the organic matter was calculated by determining the percentage of C and N in the samples in an elemental analyser. The samples were frozen after sampling and in the laboratory were freeze-dried and stored in a desiccator until the extraction of the target compounds. The extracts were concentrated in an analytical nitrogen stream, sealed in glass ampoules, and then stored in glass vials. The samples were extracted in the liquid phase by ultrasonic bath, mechanical agitation and centrifugation.

Secondly, to perform the objective two (verify the biomagnification of PFASs in tropical estuaries, figure 2) was necessary to collect 76 different specimens distributed in five trophic levels (three species of mangrove plants, sediment, suspended particulate matter, periphyton, crustaceans, molluscs and fish from different food guilds) at Subaé river. In order to evaluate the trophic position of the different organisms collected, isotopic analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was performed. $\delta^{13}\text{C}$ indicates the source of carbon by primary producers and $\delta^{15}\text{N}$ indicates, in fact, the trophic position of the organisms. The chemical compounds extraction followed the same methodology used in degradation study. With chemical analyses results and the trophic position of the organisms, it will be possible to evaluate the behaviour of PFASs in a tropical food chain, and what organisms represent a risk for consume by local people.

All project goals request laboratories and time consuming steps. Besides, because of the levels of the contaminants and complexities of the sample matrices, refined chemical analyses were necessary. They required very expensive standards and reagents, specific equipment (i.e. not available in our lab currently), expertise, and time to carry out the analyses. Moreover, all these chemical analyses were executed with Stockholm University support, which is collaborating with our project. As a result, samples were shipped overseas by a courier service (FEDEX). There are still samples to be analysed.

Time and resource were limiting factors for the development of this project that has more analytical complexity than initially anticipated. We have, nevertheless data to prepare two manuscripts. I am currently analysing the data and writing up the results to be submitted for an international journal.

3. Briefly describe the three most important outcomes of your project.

- a) All samples from objectives one (evaluate the degradation processes of sulfluramid in situ) and two (verify the biomagnification of PFASs in tropical estuaries) were collected. It is the first time that a long experiment (i.e. more than 190 day experiment) was carried out under environmental conditions with sulfluramid. The preliminary results are very exciting and

promising. Besides, until now a tropical food chain has never been studied to evaluate the behaviour of the PFASs compounds.

- b) The first in situ experiment with sulfluramid was preceded, which can generate data to promote the prohibition of this potential pollutant source. These data generated in this project could strengthen the discussion about sulfluramid uses/misuses and impacts to marine biodiversity and conservation of marine ecosystems.
- c) The project was shared with decision makers in an international meeting and got good visibility. This meeting discussed the Belém statement on Atlantic Research and Innovation Cooperation between Brazil, European Union and South Africa (see more: https://ec.europa.eu/research/iscp/pdf/belem_statement_2017_en.pdf).

4. Briefly describe the involvement of local communities and how they have benefitted from the project.

Local people were consulted on the use of sulfluramid in the region. In addition, they were involved in all field collections, indicating the best sampling sites and the most widely used subsistence organisms.

5. Are there any plans to continue this work?

Definitively, Yes! The objectives of this project discuss relevant points about the Stockholm Convention on Persistent Organic Pollutants (POPs). This data I am generating can contribute with the status changes of some compounds (i.e. PFOS) by the Stockholm Convention and, locally, giving information about the state of contamination in an environmental protection area (Todos os Santos Bay) that provide essential ecological functions, as well as source of food for several fishing communities. In addition, the problem with the sulfluramid use is getting attention by the stakeholders. Furthermore, this project is part of my PhD study that I am working in until 2021.

In summary, I intend to apply for The Rufford Foundation for a second grant to keep this project going. The costs with field and especially chemical analysis are very high and further support will be searched.

6. How do you plan to share the results of your work with others?

I already started to share the main ideas of this project. I am sharing this project in my Institution in all presentations and seminars, and, mainly, in a conference with decision makers (Belém All-Atlantic Research Forum).

1) Social media:

Facebook:

<https://www.facebook.com/photo.php?fbid=10155988812507991&set=a.488749622990.264943.649997990&type=3&theater>

2) Instituto Kimimurê: <http://www.institutokirimure.pro.br/>

3) Scientific papers:

- a. The first paper, with a provisory title "Degradation of Sulfluramide (n-ethyl perfluorooctane sulfonamide) in mangrove ecosystem" will be submitted to scientific report "Environmental Pollution"
- b. The second paper "Biomagnification of perfluoroalkylated compounds (PFASs) in a topical estuary" will be submitted to scientific report "Science of the Total Environment".

4) Academic documents: PhD thesis Daniele de A. Miranda (in progress).

5) Technical meetings: Belém All Atlantic Research Forum (<https://twitter.com/EUScienceInnov/status/1021676831118958599>)

7. Timescale: Over what period was the grant used? How does this compare to the anticipated or actual length of the project?

The RSG was used along the year fulfilling the expected duration of the project.

8. Budget: Provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion. Quotation: £ 1 = R\$ 3.85

Item	Budgeted Amount	Actual Amount	Difference	Comments
Oasis WAX 6 cc Vac Cartridge, 150 mg Sorbent per cartridge, 30 µm Particle Size, 30/pk	1050	206	-844	A methodology without cartridge was used in the most part of this project
Ammonium formate (250 g)	966	966		It was necessary to invest in the fields step before reagents
Sodium hidroxide (2,5L)	100	100		It was necessary to invest in the fields step before reagents
Formic acid (2,5 L)	337	337		It was necessary to invest in the fields step before reagents
Methanol/HPLC (4 L)	510	510		It was necessary to invest in the fields step before reagents
Acetonitrile/HPLC (4L)	214	214		It was necessary to invest in the fields step before reagents
Transportation (fuel) £ 323	323	348	+25	It was necessary to increase the

				days at field
Transportation (ferry boat rate) £ 200	200	132	-68	It was used a rented boat in the most part of this project
Alimentation £ 350	350	276	-74	It was not necessary to stay all night long on field place. The cost with alimentation was reduced
Team accommodation £ 400	400	400		It was not necessary to stay all night long on field place
Boat rent £ 300	300	807	+507	It was necessary to increase the days at field
Car rent £ 250	250	250		A borrowed car was used on the fields
Gases		1676	+1676	Nitrogen Ultra High Purity Grade Compressed (Grade 5.0) It item was necessary to reduce the samples volume to send to Stockholm University
Fridge		501	+501	It was necessary to preserve the samples until analyse
FEDEX		294	+294	It was necessary to send the samples to Stockholm University
Consumables		389	+389	This topic is in relation with stuff to work at laboratory and field (gloves, paper, screwdriver, etc.)
Total	5000		5000	

9. Looking ahead, what do you feel are the important next steps?

I need to continue with the experiments and the next important step is to concluded the first and second objectives and to initially the third objective which is to evaluate the deleterious effects that sulfluramid degradation products cause in the macrobenthic biota in the environment. This information is totally important to pressure the decision makers to get a position on banishment of the sulfluramid. We already started to discuss with the government and the scientific society about our results, and we want to do even more to achieve the mainly goal of this project, which is to contribute with the environment conservancy. It is nevertheless hard and slow to build a relationship of trust and cooperation between the university and the environmental governmental agencies, but is a crucial conservancy step.

10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

Yes! The project was presented in an important conference (Belém All-Atlantic Fórum) with more than 150 decision makers from Brazil, South Africa and European Union. This event talks about the Belém statement, with the mainly goal to do an

accord between the involved parts to protect the whole Atlantic. Moreover, in addition to my thesis, the results of this work will be shared with the scientific community through publishing peer-reviewed articles and presenting at professional conferences (example: SETAC Europe 13th Special Science Symposium)

11. Please provide a full list of all the members of your team and briefly what was their role in the project.

Daniele de Almeida Miranda: I was responsible for experimental design, field work, sample preparation, analysis, data analysis, and share the results.

Vanessa Hatje: She is my PhD advisor. She helps me to planning the field and at field work.

Josepha Manuela Leão: she is graduate student and my intern. She helps me in all field and laboratory analysis.

Raed Awad: He analysed my samples at Stockholm University

12. Any other comments?

I want to thanks the opportunity of this grant. With the support of The Rufford Foundation I could start the experiment of my thesis which is a relevant contribution for the mangrove conservancy studies.

Once I have more results and have finished data analyses, I will send in a detailed final update.



Figure 1: sulfluramid degradation experiment at tropical mangrove (Jaguaripe-Bahia, Brazil).



Figure 2: Bio-magnification experiment at tropical estuary (Subaé-Bahia, Brazil)